Bioorganic & Medicinal Chemistry 23 (2015) 6650–6658

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Rhododendrol glycosides as stereospecific tyrosinase inhibitors

Takehiro Iwadate [a], Ken-ichi Nihei [a,b,*]

[a] Department of Applied Life Science, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan
[b] Department of Applied Biological Chemistry, Faculty of Agriculture, Utsunomiya University, Tochigi 321-0943, Japan

ARTICLE INFO

Article history:
Received 26 June 2015
Revised 27 August 2015
Accepted 6 September 2015
Available online 7 September 2015

Keywords:
Rhododendrol glycoside
Phenylbutanoid
Tyrosinase inhibitor
Aldol condensation
Trichloroacetimidate glycosylation

ABSTRACT

Rhododendrol derivatives **3**–**12** have been synthesized in six steps, including aldol condensation and/or trichloroacetimidate glycosylation as the key reactions. Each derivative showed effective inhibition of tyrosinase-catalyzed oxidation processes. In particular, a series of synthetic derivatives having an *R*-stereogenic center at C-2 proved to be more potent than their respective epimers. In addition, the glycosylation on the phenylbutanoid scaffold increased the difference in activity between the isomers. This suggests that the sugar moiety plays an important role in eliciting their potent inhibitory activity.

1. Introduction

Tyrosinase (EC.1.14.8.1), known as polyphenol oxidase, is an oxidoreductase that is distributed widely in nature. It catalyzes two consecutive oxidations, that is, the *o*-hydroxylation of monophenols and the oxidation of *o*-diphenols.[1] The generated *o*-quinones can spontaneously polymerize to form various biopigments and biopolymers such as melanin. The control of tyrosinase activity is therefore considerably important in medicinal and cosmetic fields because the excessive production of melanin causes hyperpigmentation.[2–5] Tyrosinase is also responsible for the molting process of insects,[6] the infection of plant pathogenic fungi,[7] and the degradation of bioactive food polyphenols.[8] Therefore, the development of novel and effective tyrosinase inhibitors has long been pursued.[9,10]

Naturally occurring polyphenols such as flavonoids[11] and chalcones[12] often possess tyrosinase inhibitory activity. Their efficacy stems partly from a monophenolic structure that acts as a substrate analog for tyrosinase. Epirhododendrin (**1**) isolated from *Acer nikoense*[13] and rhododendrin (**2**) from *Rhododendron chrysanthum*,[14] which are classified as monophenol glycosides, are potential candidates for developing novel and water soluble tyrosinase inhibitors (Fig. 1). However, such monophenols are highly susceptible to enzymatic oxidation by tyrosinase as alternative substrates.[15–17] Conversely, several polyphenols containing the resorcinol motif are known as potent tyrosinase inhibitors that are not susceptible to oxidation.[18–22] Thus, it was envisaged that the transformation of the monophenol structures of **1** and **2** into the corresponding resorcinols might lead to novel, effective, and hydrophilic tyrosinase inhibitors.[23] Herein, we describe concise syntheses of rhododendrols **3**–**12** as well as an evaluation of their tyrosinase inhibitory activities.

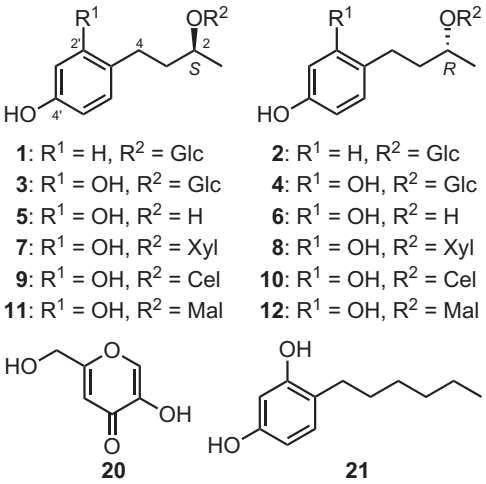
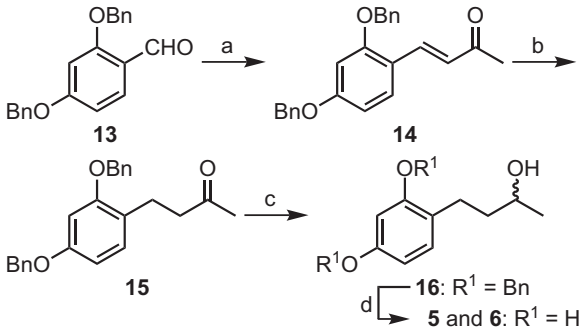


Figure 1. Structure of **1**–**12**, **20**, and **21**.

* Corresponding author. Tel.: +81 28 649 5412; fax: +81 28 649 5401.
E-mail address: nihei98@cc.utsunomiya-u.ac.jp (K.-i. Nihei).

http://dx.doi.org/10.1016/j.bmc.2015.09.014
0968-0896/



Scheme 1. Synthesis of the aglycone part. Reagents and conditions: (a) acetone, NaOH, H_2O, EtOH, 0 °C to rt, 94%; (b) H_2-Pd(en)/C, PhMe, 10 °C, 80%; (c) $NaBH_4$, EtOH, Et_2O, 0 °C to rt, 98%; (d) H_2-$Pd(OH)_2$/C, EtOAc, rt, 100%.

2. Results and discussion

2.1. Synthesis and tyrosinase inhibitory activities of 3 and 4

Benzaldehyde derivative **13**,[24] which was prepared from commercially available 2,4-dihydroxybenzaldehyde, was converted to enone **14** through aldol condensation[25] with acetone in 94% yield (Scheme 1). By catalytic hydrogenation using palladium-activated carbon ethylenediamine complex [Pd(en)/C][26] in toluene under cooling conditions (10 °C), selective reduction of **14** was achieved to produce **15** in 80% yield. When THF, EtOAc, or 1,4-dioxane was used as the solvent in this step, the reaction yield decreased to approximately 40%. Ketone **15** was transformed into alcohol **16** by hydride reduction in excellent yield (98%). Conversely, one-step synthesis of **16** from **14** was accomplished in low yield (36%) by using $NaBH_4$ in the presence of $CoCl_2$.[27]

Koenigs–Knorr reaction[28] was initially applied in the glycosylation step between **16** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide. However, the reaction proceeded sluggishly, and the isolated yield of glucoside **18** was only 12%. Using imidate **17**[29] as a glycosyl donor and $BF_3{\cdot}Et_2O$ as a Lewis acid, a complex mixture was detected on a TLC plate. Remarkably, **18** was obtained in 50% yield (Scheme 2) when trimethylsilyl trifluoromethanesulfonate (TMSOTf) was used instead of $BF_3{\cdot}Et_2O$. Resorcinol **19** was furnished in 82% yield from **18** by hydrogenolysis using Pearlman's catalyst.[30] Finally, the treatment of **19** with NaOMe produced glucosides **3** and **4** as an epimeric mixture in 89% yield. The yield of **3** and **4** was 27% over the six steps from **13**.

Table 1
Tyrosinase inhibitory activities of **3**–**12**, **20**, and **21**

Compounds tested	IC_{50}[a] (μM)	Compounds tested	IC_{50}[a] (μM)
3	4.72 ± 0.58	**4**	2.30 ± 0.15
5	2.17 ± 0.20	**6**	1.78 ± 0.10
7	4.56 ± 0.48	**8**	1.72 ± 0.17
9	3.83 ± 0.45	**10**	1.51 ± 0.10
11	4.13 ± 0.69	**12**	1.98 ± 0.17
20	9.15 ± 0.71	**21**	0.56 ± 0.02

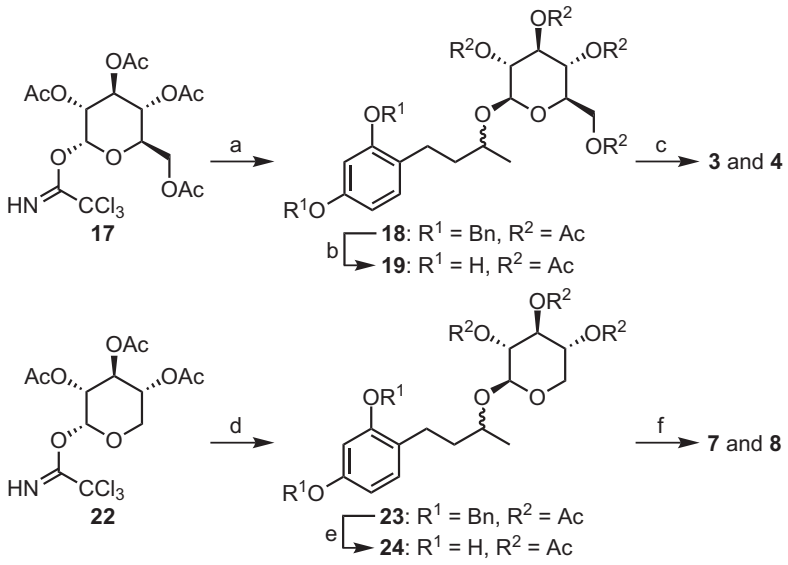
[a] The IC_{50} values represent means ± SE of three different experiments.

The separation of **3** and **4** was achieved by preparative HPLC employing conventional ODS columns.[31] The stereochemistries of the aglycone parts of **3** and **4** were determined as *S* and *R*, respectively, by comparison with previously reported ^1H and ^{13}C NMR data for **1** and **2**.[32] The evaluation of the tyrosinase inhibitory activities of **3** and **4** revealed that they showed more potent activity than that of kojic acid (**20**), a commercially used tyrosinase inhibitor (Table 1), although their efficacies were lower than that of 4-hexylresorcinol (**21**).[33] IC_{50} of the mixture of **1** and **2** could not be estimated within 100 μM.[23] Accordingly, tyrosinase inhibitory activity was significantly improved by the introduction of a hydroxy group at C-2′. In particular, the activity of **4** containing the *R*-stereogenic center at C-2 was two-fold higher than that of **3**.

The difference in activities between the epimers suggested that the rhododendrol glucosides are tyrosinase inhibitors that recognize the stereochemical environment in the enzyme structure. However, the role of the sugar moiety was still unclear. Hence, the enantiomeric pair of the aglycone was synthesized and evaluated for its inhibitory effect on oxidations catalyzed by tyrosinase.

2.2. Synthesis and tyrosinase inhibitory activities of 5 and 6

An enantiomeric mixture of **5** and **6** was prepared in 100% yield from **16** by hydrogenolysis using $Pd(OH)_2$ on carbon (Scheme 1). Unfortunately, **5** and **6** could not be separated. However, the



Scheme 2. Synthesis of glucosides **3** and **4**, and xylosides **7** and **8**. Reagents and conditions: (a) **16**, TMSOTf, CH_2Cl_2, −40 °C, 50%; (b) H_2-$Pd(OH)_2$/C, EtOAc, rt, 82%; (c) NaOMe, MeOH, 0 °C to rt, then, Amberlite IR-120H, 89%; (d) **16**, TMSOTf, CH_2Cl_2, −40 °C, 48%; (e) H_2-$Pd(OH)_2$/C, EtOAc, rt, 65%; (f) NaOMe, MeOH, 0 °C to rt, then, Amberlite IR-120H, 99%.